Terahertz and Infrared Spectra of Plumbagin, Juglone, and Menadione

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Abstract

Three naphthoquinone derivatives (plumbagin, juglone, and menadione) have been reported with antibacterial, antifungal, and antiviral properties and could potentially be used as anticancer agents because they can provoke apoptosis. However, they are also strong oxidants and cause cytotoxicity and even necrosis, so they are only limited to experimental chemotherapeutic use. In order to understand their physical chemical properties, we used both Fourier Transform Infrared Spectroscopy (FTIR) and Terahertz Time-Domain Spectroscopy (THz-TDS) to study their absorption spectra in wave number ranges of 400~4000 cm^{-1} and 6.6 ~ 92.4 cm^{-1}. We also analyzed their characteristic absorption peaks, and assigned spectra peaks in IR range to their molecular structures. These data will be very useful in future studies of their pharmaceutical mechanisms.

Introduction

Some carnivorous plants have been used as herbal medicines to cure diseases, and their therapeutic effects appear to be attributed to some naphthoquinone derivatives in the medicines. For example, a patented alternative herbal medicine Carnivora that is made from venus-flytrap *Dionaea* has been reported with immodulatory, tumorcidal, antimicrobial, antiviral, antiparasitic and antibiotic properties; and its main components include plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone) and other 1,4-naphthoquinone derivates (Keller 2001).

Plumbagin is also commonly found in *Plumbago*, *Nepenthes*, *Drosera*, and a few other plants (Jayaram & Prasad 2005; Kapadia *et al.* 2005; Likhitwitayawuid *et al.* 1998). Plumbagin and two other naphthoquinone derivates, juglone (5-hydroxy-1,4-naphthoquinone) and menadione (2-Methyl-1,4-naphthoquinone, also called Vitamin K3), have been reported with antitumor properties, since they can provoke apoptosis (Guan *et al.* 2004; Inbaraj & Chignell 2004; Parimala & Sachdanandam 1993; Sugie *et al.* 1998). However, they are also strong oxidants, leading to the formation of superoxide or oxidizing glutathione (an antioxidant, detoxifier, and immune defense system) in cells, and cause cytotoxicity and even necrosis (Inbaraj & Chignell 2004). In order to understand their chemotherapeutic mechanism before safely using them as anticancer agents, we began to use THz–Time Domain Spectroscopy (THz-TDS) to study plumbagin, juglone, and menadione; and use Fourier Transform Infrared Spectroscopy (FTIR) to reveal more physical chemical properties of these molecules. Our results may be further used in monitoring intermolecular reactions of these naphthoquinone derivatives with glutathione and other biomolecules in future.

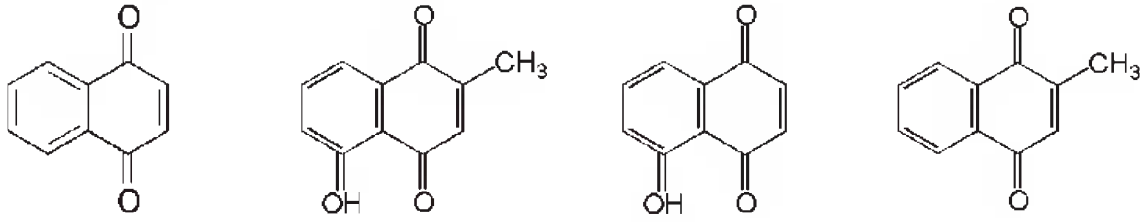
IR Spectroscopy is widely used to identify chemical compounds, because almost all organic compounds have absorption spectra in the infrared portion. IR absorption spectra can be used to distinguish functional groups of molecules and thus to determine their molecular structures (Lu & Deng 1989). IR Spectroscopy has also been used to study plumbagin (Sajan *et al.* 2005). With FTIR, we have successfully obtained the absorption spectra of plumbagin, juglone, and menadione, in the mid-wavelength IR range between 400~4000 cm^{-1}, so we compare our results with previous studies, in this paper.

THz wavelength falls within 0.1~10 THz (wave number 3.3 cm^{-1}~333 cm^{-1}), between microwaves and infrared. THz-TDS is a newly developed physical research approach, and it is highly sensitive, stable, fast, and non-ionizing and can safely penetrate through material. Thus THz spectroscopy has been quickly introduced to examine biological tissues (Mickan & Menikh 2002) and biomolecules, such as peptides, DNA, RNA, amino acids (*e.g.*, Wang *et al.* 2009), proteins, and other biomolecules (*e.g.*, Wang *et al.* 2005). Yet, it provides access to THz region that is difficult to reach by conventional means, and can be used to supplement Fourier Transform Infrared Spectroscopy (FTIR). With THz-TDS, we have successfully obtained THz spectra of plumbagin, juglone, and menadione, in the range 6.6~92.4 cm^{-1} (0.2~2.6 THz). In this paper we will present our results and discuss the possible correlations between specific absorption peaks and molecular structures, using both FTIR and THz-TDS approaches.

Material and Methods

All samples (see Figure 1) were obtained from Sigma-Aldrich Chemical Co. (USA), plumbagin (98%) in orange yellow polycrystalline grains, juglone (97%) in yellow needles, and menadione (98%) in yellow polycrystalline powder. For FTIR the samples were mixed with potassium bromide in a mass ratio 1:10, while samples for THz-TDS were mixed in a mass ratio 2:1 with polyethylene powder that is nearly transparent for THz wave. Then they were pressed under a pressure of 1500 kg and 2000 kg respectively into disks 1.3 mm in diameter and 1 mm in thickness.

Infrared spectra were recorded with Equinox 55 FTIR spectrometer of Bruker (Germany). The resolution is 4 cm^{-1} and the bandwidth is 4000–400 cm^{-1}. The THz-TDS system was set up as illustrated in Figure 2. A mode-locked Mai-Tai Laser of Spectra-Physics (USA) was used to generate laser pulse. The system has a sample box that is purged with dry nitrogen to keep the humidity less than 2%, to minimize the vapor effects and thus enhance the signal-to-noise ratio (SNR). Within the sample box are an InAs wafer with <100> orientation to emit THz beam and a ZnTe wafer with <110> orientation to receive signals. Sample signals were yielded when a THz beam passed through a sample between the two wafers, and reference signals were received when the THz beam passed



Naphthoquinone Plumbagin Juglone Menadione

Figure 1: Structural formula of naphthoquinone (1,4-naphthoquinone), plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone), juglone (5-hydroxy-1,4-naphthoquinone), and menadione (2-Methyl-1,4-naphthoquinone).

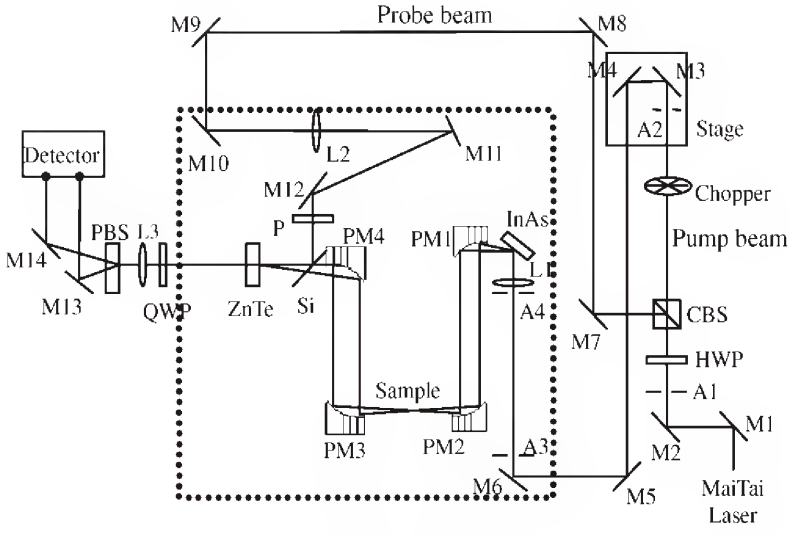


Figure 2: Diagram of the THz-TDS system

through the box without sample. Both reference and sample signals were compared and calculated automatically to plot spectra with reflection index and absorption coefficient.

Results

Figure 3 shows the absorption spectra of plumbagin, juglone, and menadione in the IR range between 400~4000 cm^{-1} with many characteristic peaks that can be easily assigned to their functional groups or structures (Table 1), based on previous studies (Chawla *et al.* 1995; Globus *et al.* 2004; Lu & Deng 1989). For example, absorption peaks of C=C are found in the range 1690-1635 cm^{-1} (Lu & Deng 1989).

The THz absorption spectra of plumbagin, juglone, and menadione were obtained, with a validate range between 0.2~2.6 THz (see Figure 4), and their absorption peaks are presented in Table 2. Although they all belong to 1,4-naphthoquinones derivatives, their THz spectra obviously have characteristic absorption peaks, and thus can be used as molecular fingerprints in identification.

Discussion

For IR spectra, we will mainly discuss the characteristic absorption peaks of three functional groups (C=O, OH, and CH_3) of plumbagin, juglone, and menadione, in following five aspects:

Table 1. Assignments of functional groups and structures of plumbagin, juglone, and menadione.

	Plumbagin cm^{-1}	Juglone cm^{-1}	Menadione cm^{-1}
V(C=O)	1664.16 1644.09	1665.41 1643.35	1665.07 1622.22
V(OH)	3436.69	3435.88	
V(CH_3)	1365.14		1379.31
δ (ring)	1608.69	1600.24	1593.01
V(CH) on the ring	2964.24	3071.41	3068.33 2957.74
β (CH)	1303.59 1258.75	1337.13 1290.49	1327.57 1301.10
γ (CH) on the ring	835.93 753.22	858.11	779.89

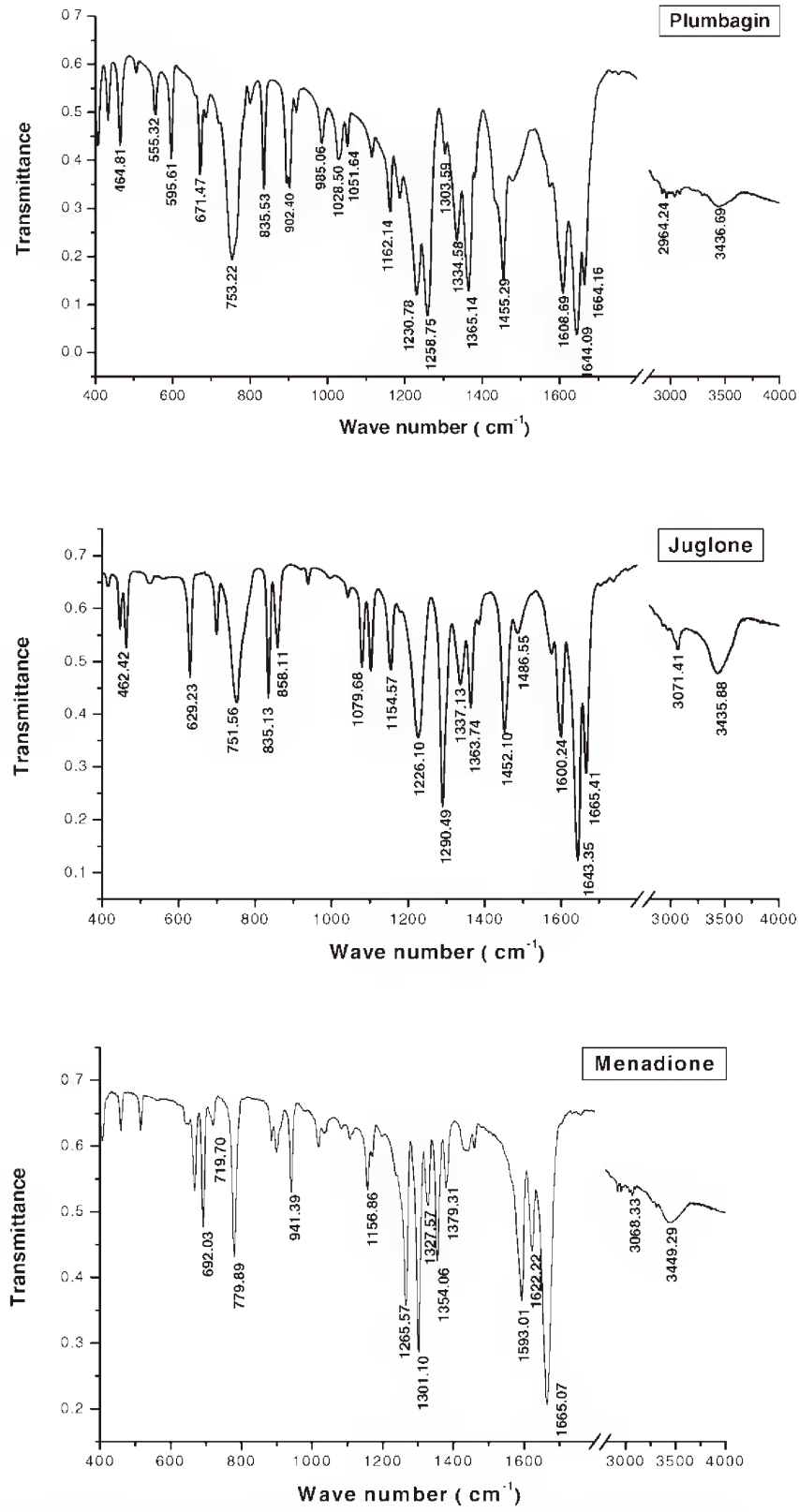


Figure 3: IR Absorption spectra of plumbagin, juglone, and menadione.

1) The hydroxyl group (OH) of juglone and plumbagin occurs at 3435.88 cm^{-1} and 3436.69 cm^{-1} respectively (Table 1), closely matching with the OH peak (3412 cm^{-1}) of plumbagin reported by Sajan *et al.* (2005).

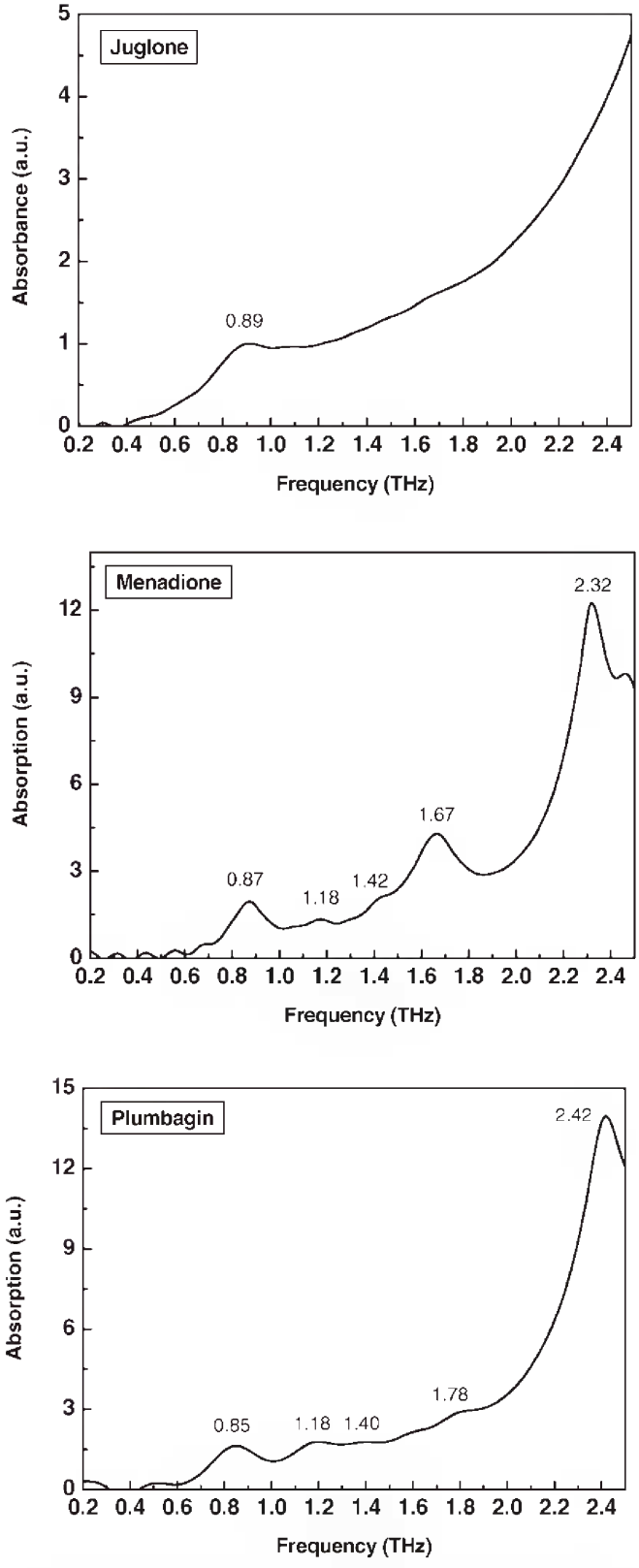


Figure 4: THz absorption spectra of plumbagin, juglone, and menadione.

2) Different from juglone, the IR spectra of menadione and plumbagin have a strong absorption peak between 1360 cm^{-1}~1380 cm^{-1}, due to existence of methyl group (CH_3). In other words, the existence of peaks at 1379.31 cm^{-1} and 1365.14 cm^{-1} indicates the existence of the methyl group (CH_3) on the aromatic ring (Lu & Deng 1989; Sajan *et al.* 2005).

3) Commonly, a carbonyl group (C=O) of quinones has 1-2 characteristic absorption peaks between 1690 cm^{-1} and 1635 cm^{-1}. When a carbonyl group (C=O) has two peaks, the higher frequency peak would be normally stronger than the lower frequency peak. However, when there is a hydroxyl group (OH), the higher frequency peak would become weaker (Lu & Deng 1989). In our results, plumbagin, juglone, and menadione all have two peaks within the frequency range, and the peak strengths really concur with the aforementioned statements: the 1665.07 cm^{-1} peak of menadione is stronger than its 1622.22 cm^{-1} peak; and the higher frequency peak of juglone and plumbagin (both have a hydroxyl group) is weaker than their lower frequency peak (see Figure 3).

4) When a carbonyl group (C=O) has two peaks, and if there is a hydroxyl group on the aromatic ring, the frequency of the lower frequency peak would be decreased (Lu & Deng 1989). Both plumbagin and Juglone have a hydroxyl group (OH), however, our results show that their lower frequency peaks have higher frequency at 1644.09 and 1643.35 cm^{-1} respectively than the 1622.22 cm^{-1} peak of menadione that lacks a hydroxyl group (Table 1). These results are very different from previous conclusions and should be further studied.

5) Interestingly, the absorption spectrum of plumbagin reported by Sajan *et al.* (2005) showed only one absorption peak at 1663.69 cm^{-1}, but now we have found two peaks at 1664.16 cm^{-1} and 1644.09 cm^{-1} for carbonyl group (Table 1).

Table 2. THz Absorption peaks of plumbagin, juglone, and menadione (Frequency unit: THz).					
Plumbagin	0.85	1.18	1.40	1.78	2.43
Juglone	0.89				
Menadione	0.87	1.18	1.42	1.67	2.32

In Sajan *et al.* (2005) the peak around 1644 cm^{-1} could be masked by a very strong peak at 1608.54 cm^{-1} of the δ ring, while in our spectrum the δ ring peak at 1608.69 cm^{-1} is much weaker and the 1644 cm^{-1} peak is the strongest (see Figure 3). Sajan *et al.* (2005) also obtained plumbagin from the same company, but with 99.9% purity. Maybe the higher purity strengthened the absorption peak of the δ ring.

The THz absorption peaks of large molecules are commonly interpreted as collective rotational and vibrational modes of the whole molecules, determined by their molecular configuration and conformation (Siegel 2000; Mickan & Zhang 2003), rather than referring to specific functional groups/structures. However, since all three 1,4-naphthoquinone derivatives have most parts identical to each other, their common absorption peaks should be determined by their basic, common molecular structures, while their different peaks could be correlated with their specific functional groups. On this basis, we have comparatively analyzed the THz spectra of plumbagin, juglone, and menadione, and found some correlations between absorption peaks and molecular structure and functional groups, in following three aspects:

1) Plumbagin, juglone, and menadione all have a basic 1,4-naphthoguinone structure (see Figure 1), and all have an absorption peak between 0.85~0.89 THz (see Figure 4, Table 2). This peak appears to represent a rotational and vibrational mode that is determined by the basic molecular structure.

2) Plumbagin and juglone are almost identical (see Figure 1), but plumbagin has a methyl group (CH_3), and its THz spectrum has four more absorption peaks (see Figure 4, Table 2). Therefore, the four additional peaks appear to be correlated to the methyl group. The above hypothesis has been supported by menadione that also has a have a methyl group (CH_3) at the same position (see Figure 1), and menadione also has four additional absorption peaks. The four additional peaks of both plumbagin and menadione are very close in terms of frequency and strength (see Figure 4, Table 2). Therefore, increased absorption peaks appear to be related to the addition of the methyl group (CH_3) that could cause dynamic changes of the molecules; and the same correlation is also found in our study of amino acids (Wang *et al.* 2009). The addition of a methyl group to the 1,4-naphthoquino structure could increase the intermolecular hydrogen bond vibrations, as Fischer *et al.* (2002) suggested based on their study of THz spectra of DNA bases, and thus cause the increase of vibrational modes that are represented by absorption peaks.

3) Comparing the THz spectra between plumbagin and juglone, both have a hydroxyl (OH) group (see Figure 1), plumbagin has five absorption peaks, while juglone has only one absorption peak (see Figure 4, Table 2). Therefore, the hydroxyl group itself appears not a factor causing the different absorption peaks. On the other hand, menadione does not have a hydroxyl group, but it also has five absorption peaks. So the presence or absence of hydroxyl group would not affect the absorption spectra in this THz range.

In short, many of results of these FTIR and THz spectra of plumbagin, juglone, and menadione, are reported for the first time. These important data can be further used to study the pharmaceutical mechanism of these 1,4-naphthoquinone derivatives and their interactions with biomolecules.

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